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Bacteriological quality of broiler chicken meat sold at local market of Kanchanpur district Nepal

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ABSTRACT
Chicken is an excellent source of good quality protein, but it is highly susceptible to microbial contamination and often implicated in food borne disease. The microbiological quality of chicken at different local markets of Kanchanpur district of Nepal was investigated during the March 2014 to April 2015. This cross sectional study included 45 samples of fresh chicken meat from all five municipalities of Kanchanpur were collected and analyzed for total viable count, indicators and pathogens of meat. The streak plate technique was employed for enumeration and identification bacteria. The mean of total viable count in the chicken of Kanchanpur district ranged from 8.76 Log_{10}cfu/g to Log_{10}11.46cfu/g with mean Log_{10}10.17 ±0.1cfu/g. The mean of TVC in the chicken of Bhim Datta Municipality, Belauri Municipality, Dodhar Chandani Municipality, Jhalari Pipaladi Municipality and Punarbash Municipality were Log_{10} 10.46±0.3cfu/g, Log_{10} 9.97±0.3 cfu/g, Log_{10}10.32±0.2 cfu/g, Log_{10}9.53 ±0.2cfu/g and Log_{10}10.45±0.3 cfu/g . A five types indicator and food borne pathogens 18 (40%) of *E. coli*, 17(38%) of *Shigella spp*., 17(38%) of *Salmonella spp*., *Citrobactor spp.,* and 26 (57%) of *Staphylococcus aureus* were isolated and identified from samples. The retail broiler meat samples from the locations contain over the permissible limit of bacteria suggesting deplorable state of hygienic and sanitary practices. The presence of *Salmonella* and *Staphylococcus aureus* organisms over the permissible limits are of special concern because these account for potential food borne intoxication.
Key Words: Bacterial quality; chicken; total viable count; total coliformm, *Salmonella spp.*

INTRODUCTION

Chicken is an excellent source of good quality protein but it is highly susceptible to microbial contamination and often implicated in food borne disease[1]. The presence of pathogenic bacteria in meat indicates public health hazard and give warning signal for the possible occurrence of food borne intoxication [2]. Outbreaks of food-borne diseases have led to considerable illness and even death [3]. Meat borne diseases such as Salmonellosis, *E. coli* enteritis and food poisoning by *Clostridium, Staphylococcus*, etc. are the major problems caused by eating contaminated meat [4].

Broiler meat is now considered as very cheap source of protein for the consumers which must be safe to devour. Microbial food safety issues are becoming more important in international trade [5]. In Nepal, increasing population and growing income level of consumers have added to an increased demand for livestock and poultry products [6]. The meat quality is adversely affected by careless handling conditions in the slaughtering places as well as in the meat markets or shops [7]. Some butchers or processors are slaughtering and processing the poultry in their premises with poor hygienic condition [8]. High majority of consumers buy meat from butcher's shop at which food hygiene and safety condition are not assured and there is no information on prevalence of *Salmonella* and other food borne infection in retail meat shops in Nepal [9].

In developing countries like Nepal, lack of appropriate slaughtering facilities, techniques and meat inspection are causing contamination of meat by pathogens including zoonotic parasites [7]. Chicken meat can be contaminated at several points throughout the processing operations. Such contamination may render the chicken meat unsafe to consumer or impair its quality [10]. The presence of *Salmonella* and *Stphylococus aureus* organisms over the permissible limits are of special concern because these account for potential food borne intoxication. So, the need for microbial assessment of fresh meats can be emphasized [2]. The available epidemiological data about food-borne illness suggest that broiler meat consumption is still the primary cause and one of the main sources of food-borne infections in humans [11]. Hence, this study was conducted to assess the microbiological situation of fresh chicken meat.

MATERIALS AND METHODS

Study Area

A cross-sectional study was conducted for assessment of microbial quality of chicken meat during the month of March 2014 to April 2015. A purposive sampling method was used for sample collection in five Municipalities of Kanchanpur district. Kancharpur district is 5 km east of the Indian border and 700 km west of Kathmandu. It covers an area of 1,610 km^2 and had population 451,248 [12]. Politically a Kanchanpur district is

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divided into five municipalities and thirteen village development committees. Local markets of five municipalities such as Mahendranagr bazaar of Bhimdatta Municipality, Dodhara and Chadani bazaar Dodhara Chadani Municipality, Jhalari bazaar of Jhalari Pipladi Muncipality, Belauri bazaar of Belauri Municipality and Kalika, Punarbas bazaar of Punarbas Muncipality were selected from the district where broiler population density is comparatively high. These places are from all four direction of the district which can easily represent the Kanchanpur district. Also, these areas are easily reachable and large numbers of customers of commissioner of this research are concentrated.

Sample Collection

A forty five fresh chicken meat were purchased from a local retail store of five municipality local towns namely Mahendranagar, Dodhara-Chadani, Belauri, Kalika, Punarbas and Jhalri of Kanchanpur district. Forty five chicken samples were collected from the meat shop those being selling chicken at the time of researcher arrived of above local market. Consents of the shopkeepers were taken before moving to the collection of the samples. Freshly slaughtered 25g meat samples from the above outlets were collected into sterile plastic bags, labeled store at 4^{o}C in ice chest filled with ice and transported to the Microbiology laboratory of SNSC for immediate analysis.

Microbiological Analysis

The complete process of microbiological analysis was performed under a sterile condition. The samples were subjected to microbiological analysis according to standard procedures [13]. Chicken meats were analyzed following the standard microbial method using Hi-media culture as supplied by HIMEDIA® Pvt Ltd., India. Samples (25g each) were homogenized in 225 ml lactose broth (LB) using blender for 2 min at high speed. Thus 1:10 dilution of the sample was obtained and the homogenate was then serially diluted and spread plated (0.1 ml) on a range of enumeration agar plates: MacConkey agar for coliforms; Eosin Methylene Blue (EMB) for *E. coli*; Manitol Salt Agar (MSA) for *S. aureus*; Salmonella-Shigella agar for *Salmonella*; The total viable count was performed by standard plate count method used Potato Dextrose Agar. The agar plates were incubated at 37^{o}C for 24 hours after which colonies were counted using Quebec Dark field colony counter. Plates with approximately 30-300 colonies were selected for counting of results. The plate count was exceeded 300 colonies than further serial dilutions and plating was immediately carried out within 24 hours on dilutions stored in a refrigerator. The results of the total bacterial count were expressed as the number of colony forming unit per gram (cfu/g) of meat samples.

Characterization and Identification of Isolates:

Bacteria isolates were characterized and identified after studying the Gram reaction as well as cell micro morphology. Other tests performed were spore formation, motility, oxidase and catalase production; citrate utilization, Oxidative/Fermentation (O/F) utilization of glucose; indole and coagulase production, starch hydrolysis, sugar fermentation, methyl red, Voges-Proskaur reaction and urease production. The tests were performed according to the methods of [14].

Statistical Analysis

Quantitative and qualitative information of types and bacterial load were collected by Microbiological laboratory investigation of chicken by culture and bio-chemical identification. The recorded data were tabulated using Microsoft Excel 2007. The means were calculated for total viable organism from total plate counts. All bacterial counts were expressed as $\log_{10}$ colony forming units per gram ($\log_{10}$ cfu/g). The mean $\log_{10}(x)$ value and standard error were calculated on the assumption of a log normal distribution. Mean obtained of total viable counts were compared with different municipality by One Way of Analysis of Variance (ANOVA) for each sites to determine difference in group means. Mean of TVC of different sites were also compared to each other by two tailed t- test to determine the difference in between the two mean. Descriptive statistics was used to describe the result of prevalence analysis. Prevalence was estimated as the number of samples detected positive to *E. coli*, *Salmonella spp*., *Shigella spp., Enterobactor spp.*, *Citrobactor spp., Staphyllococcus aureus i*solation from the total sample analyzed. The chi-square test was used to assess any statistical significant association between above isolated microorganism with regard to isolated of bacteria from chicken meat.

RESULT

A total of 45 chicken meat samples were collected and analyzed for assess the microbial quality of chicken meat in Kanchanpur district. Five municipalities were selected for study site. Fresh chicken meat samples were collected from local market open meat shop from each municipality.

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Microbiological Quality of Chicken Meat

Table 1: Total Viable Counts of Broiler Meat in log_{10} CFU/ gram ± Standard Error (SE)

Study Site	Mean TVC	Range of TVC
Bhim Datta Municipality (n=11)	10.46±0.3[a]	8.78 -11.46
Belauri Municipality (n=8)	9.97±0.3[a]	8.76 - 11.45
Dodhara- Chad ani Municipality (n=10)	10.32±0.2[a]	9.24 - 11.46
Jhalari-Pipladi Municipality (n=8)	9.53 ±0.2[a]	8.88- 11.11
Punarbash Municipality (n=8)	10.45±0.3[a]	8.95 – 11.42
Total (n=45)	10.17±0.1	8.76 – 11.46
F-value	Calculated=0.757 Tabulated=2.61	

Key: TVC= Total Viable Count

[a] Means with the same letters in a row were not significantly different (calculated t-values are smaller than tabulated). The ANOVA, the calculated f-value is smaller than tabulated value showed that there was no significant difference in the mean of total viable count.

Table 2. Frequency distribution of bacteria isolated from fresh chicken meat

Pathogen	Total (N=45)		BDM (n=11)		BM (n=8)		DCM (n=10)		JPM (n=8)		PM (n=8)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
E. coli	18	40	6	55	0	0	7	70	2	25	3	38
Salmonella spp.	15	33	7	64	1	13	3	30	0	0	4	50
Shigella spp.	17	38	2	18	3	38	3	30	5	63	4	50
Enterobactor spp.	5	11	3	27	1	13	0	0	0	0	1	13
Citrobactor spp.	15	33	1	9	2	25	3	30	6	75	3	38
Staphylococcus aureus	26	57	8	73	4	50	6	60	2	25	6	75

P- Value >0.05

Note: BDM= Bhim Datta Municipality, BM= Belauri Municipality, DCM= Dodhara Chandani Municipality, JPM= Jhalari Pipaladi Minicipality, PM= Punarbash municipality

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Forty five chicken meat samples from local market of five municipalities of Kanachpur district were analyzed microbiologically for the incidence of meat borne pathogen and indicator bacteria. The mean of TVC of chicken meat of Kanchapur district was $\log_{10}$ 10.17 cfu/g with range $\log_{10}$ 8.76- 11.46 cfu/g (Table 1). Among the five municipalities, the highest mean of TVC was found $\log_{10}$10.46 cfu/g followed by Punarbash Municipality $\log_{10}$10.45cfu/g and Dodhara- Chadnani Municipality $\log_{10}$10.32 cfu/g which were higher than the mean of whole district also. The mean TVC was found $\log_{10}$ 9.97cfu/g in Belauri Municipality and lowest mean of TVC was $\log_{10}$9.53 cfu/g of chicken meat.

In the present study, the mean of the TVC of broiler chicken meat of all five municipalities and Kanchanpur district were found over the permissible limit. TVC permissible limit by ICMSF, 1986 is log10 7 cfu/g for raw chicken. Since, no such standards have been recommended for the permissible limit of raw poultry meat in Nepal. The mean of TVC of fresh broiler in Bhim Datta Municipality, Belauri Municipality, Jhalari Pipladi Municipality and Punarbash Municipality did not significantly differ. The ANOVA test was performed and found that there was no significance difference of the mean count of total viable count of broiler chicken meat. These statistical tests suggested the total viable count of bacteria did not depend on geographical differences.

Bacteriological isolation was performed for all 45 chicken meat samples and isolated colonies were identified (Table 2). Over all bacteria, *Staphylococcus aureus* was found in highest 26 (57%) of samples. *E. coli* was isolated from18 (40%) samples followed by *Shigella spp* was in 17(38%), *Salmonella spp.* and in *Citrobactor spp.* in 15(33%) samples. The *Enterobactor spp.* was found in lowest 5(11%) of samples. The chi-square test was performed for the isolated. The statistical result showed that there was no significant difference ($P>0.05$) of bacteria present in meat sample.

The *Staphylococcus aureus* was found to dominants all over the isolated organism in most of the municipalities. The highest prevalence was found 6(75%) in PM and lowest 2(25%) in JPM. *E. coli* was isolated highest 7(70%) in samples of DCM and lowest 2(25%) in JPD. It was not isolated from Belauri municipality. The chicken meat samples 7(64%) of BDM showed highest contamination with *Salmonella spp*. and lowest 1(13%) in BM. It was not found in JPM. The very low prevalence of *Enterobactor spp.* was found in municipalities but highest 3(27%) were found in BDM and lowest 1(13%) were BM and PM. *Enterobactor spp*. was not isolated from meat samples of both DCM and JPM. *Citrobactor spp.* was found highest 6(75%) in samples of JPM and lowest 1(9%) in BDM.

DISCUSSION

Total Viable Count

The broiler fresh chicken meat of Kanchanpur district was found high microbial count over than permissible limit. TVC permissible limit by ICMSF, 1986 is $\log_{10}$ 7 cfu/g for raw chicken. The result was lower than those obtained in local chicken market Bharatpur Municipality and Ratnanagar Municipality of Chitwan district of Nepal.[2] The result was higher than those found mean of APC was $\log_{10}$4.01cfu/g of chicken meat at commercial processing plant near Lahore, Pakistan.[15] Similarly, the TVC was also greater than that found $\log_{10}$ 6.91cfu/g in chicken meat samples collected from local market at Accra in Ghana.[16] And chicken meat samples of Bangalore in India.[17] Similar study carried out the work in Pondicherry (India) to find and compare the differences in quality of fresh chicken obtained from market/road side chicken shop ($\log_{10}$ 6.28 cfu/g) was also lower than present study.[18]

The ANOVA, F -value (calculated value is less than tabulated values) showed that there was no significant difference in the mean counts of the total viable count. The calculated t-values were also smaller than tabulated values of the mean of TVC of all five municipalities thus this statistical value suggest that there was no significantly difference between the mean of TVC. The variation of microbial count in chicken sample did not depended on location but there may be another contribution factors that contributed the microbial contamination of chicken meat. The high microbial count enumerated from fresh chicken meat samples indicated that the meat samples were contaminated. Microorganisms may be able to easily be introduced either in the pre or post processing stages of meat processing.

This observation could be largely high it may due to the use of improved and poor hygienic practices during slaughtering and marketing of meat is one of the major contributing factors for unsafe meat in Nepal. Slaughtering animals in open and unhygienic places, use of dirty water during slaughtering process, and selling meat in open and non-refrigerated places are some of the unhygienic

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practices being used by the entrepreneurs. Slaughtering of animals in unhygienic place and use of polluted water contaminate the meat with different microorganism which can be harmful for the health of the consumers. Total viable count was indicative of the populations of spoilage microorganisms and act as an index of hygienic quality.

Pathogens Isolated from Chicken Meat

Fresh chicken meat samples from five locations (BDM, BM, DCM, JPM and PM) yielded marked growth of bacteria. The presence of these organisms on meat parts could be attributed to the fact that meat contains an abundance of all nutrients required for the growth of bacteria in adequate quantity. The high total viable counts recorded in this study showed the microbial diversity (differences in form or species) in these markets, condition of the market and the hygienic practice employed by meat sellers and butchers. This determined the variation of bacterial contamination.

A total of six genera of pathogen and indicator bacteria were isolated and identified from 45 samples. The bacteria isolates were identified as *Enterobacter spp., Citobacter spp., E. coli, Salmonella spp., Shigella spp.* and *Staphylococcus aureus* by comparing their morphological and biochemical characteristics with standard reference organisms [14]. The bacterial contamination of meat samples showed no significantly differences (P>0.05). The result showed that chicken meats were often contaminated with microorganisms due to unhygienic and poor sanitary conditions. Microorganisms isolated from fresh meat samples in this study have been earlier found in foods, environment and other places. The presence of these organisms in fresh meats depicts a deplorable state of poor hygienic and sanitary practices employed in the slaughtering, Moreover, the faecal coliforms as *Escherichia coli* are generally considered as indisputable indicators of faecal contamination from warm blooded animals [4].

The presence of *E. coli* (40%), and *Enterobacter spp* (11%) in this fresh meat samples is an indication of faecal contamination of the meat. However, the finding of this study was compared to a study undertaken to Kolkata and found that the *Staphylococcal* and *E. coli* counts were higher in chicken meat from urban markets than semi-urban markets.[19] The another study conducted in wet market of Bangalore reported the prevalence of *Salmonella* in the range of 25 to 65% and *E. coli* in the range of 42 to 88 %. [17] *Escherichia coli* 48.4% from the poultry meat samples in Casablanca (Morocco).[20] And 60.63% *E. coli* were reported.[21] These findings are higher than the present study. Similar study was also conducted in Washington DC, US and isolated 82 (38.7%) *E. coli* from the 212 chicken samples which was lower than present study [22]. *Escherichia coli* are an enteric organism and its presence is an indication of faecal contamination of the samples. This may attributed to improper sanitary condition during processing of the meat from the water supply contaminated utensils and contamination by flies causes gastroenteritis in infants and young children [23].

According to this study, prevalence of Salmonella in retail broiler meat in Kanchanpur district was found to be 38% which is higher than that obtained than previous study [24]. The another study conducted in Chitwan Valley 26.1 % of *S. typhimurium* were isolated [25], and 14.6% of *S. typhimurium* were isolated from total 82 samples of poultry related products in Namakkal district of Tamil Nadu [26]. However, this prevalence rate is lower than that of found 46.2 % Salmonella in poultry meat in Chitwan [2]. The another study conducted in Crotian market of Zagreb found that 10.46% *Salmonella* and 30.30% *Staphylococcus aureus* in chicken meat at which are lower than present study [27]. This difference can be related to difference in time and season of research from those researches. The presence of *Salmonella* and *Staphylococcus aureus* organisms demonstrates a potential health risk since the organisms are pathogenic and gives warning signal for the possible occurrence of food borne intoxication. This study revealed the prevalence of *Staphylococcus aureus* was 57% highest among the other pathogen in all study sites. This study showed higher prevalence of *S. aureus* than another study [27]. In another study 90.63% *Staphylococcus aurus* was isolated which was higher than present study [21].

The reason for the high prevalence of *S. aureus* could have been the poor personal hygiene of the workers and the technique used for opening the abdomen practiced in the traditional shops. Such a high level of contamination with of meat *S. aureus* has been associated with increased risk of staphylococcal food poisoning [28]. *Staphylococcus aureus* which is a normal flora of the body indicates contamination from handlers. The organism can pass onto food during harvesting, processing or even storage. It is the major cause of food poisoning known as staphylococcal food poisoning. The poisoning is caused by the ingestion of an

enterotoxin produced, which is characterized by diarrhea and vomiting [29].

The prevalence Gram negative bacteria *Shigella spp*. was in 17(38%), *Citrobactor spp.* in 15(33%) samples and *Enterobactor spp.* was found in lowest 5(11%) of samples in the present study. *Enterobacteria spp.* 34.84% were isolated from chicken which is higher than this study [28].The presence of these organisms in the chicken meat is indicative of public health hazard and gives a signal of the possible occurrence of food borne intoxication and infection. This also implies that these meats are viable source of various diseases. Some of these diseases could spread and acquire epidemic status which poses serious health hazards. The higher counts in local samples were may suggest poor quality issue and possible poor temperature control. Undoubtedly the poultry slaughtered and dressed under local conditions in Kanchanpur, may carry high initial contamination bacterial load from the point of slaughtering process to the point of offering to consumers. There may occurred biomagnifications at all levels of handling, poor transport and retailing conditions.

It is therefore necessary to explore the use of an innovative food processing technology such as irradiation in addition to traditional temperature management techniques such as chilling and freezing. In particular, the technology of irradiation has been recognized as one of the safest and most effective methods for inactivating bacteria in raw poultry either freshly chilled or frozen.

CONCLUSION

The marked growth of bacteria concludes that broiler chicken meat is not suitable for consumption. The high total viable counts recorded in this study showed all the samples were over permissible level of microbial load and the microbial diversity (differences in form or species) in these places, condition of the market and the hygienic practice employed by meat sellers and butchers. The presence of *Salmonella, Shigella, E. coli, Enterobactor, Citobacoter and Staphyloccus aureus* organisms demonstrates a potential health risk since the organisms are pathogenic and gives warning signal for the possible occurrence of food borne intoxication. The need for microbial assessment of fresh meats and other meat products processed and packaged for human consumption is therefore emphasized and recommended to reduce possible hazard. It is concluded that contamination of poultry and poultry products should be prevented during handling, slaughter and processing to protect the public from infections and diseases.

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